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Award Number: W81XWH-10-1-0496

TITLE: Response of human prostate tissue to hypofractionated ionizing radiation.

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REPORT DATE: May 2012

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
May 2012	Annual Summary	1 July 2010 – 30 April 2012

4. TITLE AND SUBTITLE

Response of human prostate tissue to hypofractionated ionizing radiation.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0496

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Arizona
Tucson, Arizona 85719-4824

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The aim of this proposal was to determine the differences in radiobiological response of human prostate tissue to conventional and hypofractionated radiotherapy. Specifically, this proposal characterized the predominant DNA damage response pathway from human prostatectomy specimens in response to a conventional or hypofractionated dose of ionizing radiation. We demonstrate that normal prostate tissue and prostate cancer can be cultured ex vivo using a dynamic culture system and used to study the radiobiology of human prostate tissue. Normal prostate tissue responds to ionizing radiation with predicted repair foci (gamma-H2AX and Rad51), and a marker for cellular stress, p53. The DNA damage response in normal glands appears to predominate in the basal cell layer. Prostate cancer epithelium responds to ionizing radiation with predicted early repair foci (gamma-H2AX), but in contrast to normal prostate epithelium, homologous repair foci (Rad51) were not demonstrated. The ex vivo organ culture technique will be optimized to mimic the indolent nature of prostate tissues and retain the integrity of the stroma. This system will promote testing of clinically relevant questions for translational prostate cancer research.

15. SUBJECT TERMS
Human Prostate Organ Culture, Ionizing radiation, Hypofractionation, DNA damage repair

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	23	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

The goal of this training grant was to provide specialized clinical training and research experience in prostate cancer. My endpoint for success at the completion of the award was to build on the training reported within and begin a career as a physician-scientist in the discipline of Radiation Oncology with a focus on prostate cancer. This endpoint has been met successfully (Reportable Outcomes).

The training component of the grant includes basic science and clinical mentorship, specialized training in prostate cancer radiotherapy, scientific writing, presentation at national meetings, and career development sessions. From a basic science standpoint, the goal was to build upon the skills obtained in graduate school to be successful in the laboratory and refine the art of scientific writing and presentation. From a clinical perspective, a focus on specialized training in prostate cancer radiotherapy not offered at my institution was desired. The endpoint of completing the training as Board Eligible in Radiation Oncology has been obtained (Reportable Outcomes).

The research component of the grant is the proposal to determine the differences in radiobiological response of human prostate tissue to conventional and hypofractionated radiotherapy using a unique *in vitro* model system for culturing tissue obtained from prostatectomy specimens. The primary radiobiological endpoint is to determine the predominant DNA damage response pathway involved in repair of double-strand breaks in both the epithelial and stromal components of human prostate tissue. This proposal has clinical significance because studying the DNA damage response of human prostate tissue can uncover unique response signatures that may serve as biomarkers for clinical outcome after radiotherapy. The research reported within has been presented at national meetings and has a manuscript in preparation (Reportable Outcomes).

BODY:

Training Program:

Focus Area 1: Mentor Guidance.

Dr. Anne E. Cress has served as mentor on this grant. During the funding period she and I have weekly meetings to discuss the research project. Our meetings focus on technical assistance, data analysis and future directions of the project. We have met after each scientific and technical conference I have attended during the funding period to discuss relevant abstracts and brainstorm new research avenues based on topics discussed at national meetings. I have been mentored on abstract preparations (Reportable Outcomes) and given an opportunity to present my data prior to national meetings at the Cress laboratory meeting.

Dr. Cress is the Deputy Dean for Research and Academic Affairs at the College of Medicine. Therefore, with her expertise, she has been able to provide me with specific mentorship regarding career development and planning for research independence. She has been a member of NIH study sections and we have had in-depth discussions on successful grant preparation. She is currently mentoring me on a grant to be submitted for

a Prostate Cancer Physician Training Award through the Department of Defense (Reportable Outcomes).

Given my project was dependent on collection of fresh human prostatectomy specimens which can vary in their availability, Dr. Cress has encouraged translating the core concepts of my research project into cell line based work in order to increase scientific efficiency. This has resulted in additional abstracts submitted during the funding period (Reportable Outcomes).

Focus Area 2: Clinical Prostate Cancer Training.

Treatment of prostate cancer as a radiation oncologist requires knowledge and expertise of both external beam radiation and brachytherapy. Brachytherapy is a specialized technique in which a physician places radioactive seeds within the prostate. An essential part of the training program was to obtain training in this technique. In September 2010 I attended the Ultrasound-Guided Transperineal Brachytherapy course and in June 2011 I attended the High Dose Rate Brachytherapy course, both were offered through the Seattle Prostate Institute. The programs focused on all aspects of brachytherapy including: patient selection, physics and dosimetry planning, technique, quality assurance, complications, setting up an implant program, post implant dosimetry and patient follow-up.

During the funding period I was awarded the Seattle Prostate fellowship through the American Brachytherapy Society. This provided me with a two week training program with experts in ultrasound-guided transperineal brachytherapy. I participated in the fellowship in December 2011. I received hands-on training in this technique. This was a competitive award and a critical step from a clinical perspective to becoming a prostate cancer expert.

Throughout the granting period I was involved in quality assurance rounds weekly. This provides a venue to critically review patient radiotherapy plans. We review both external beam and brachytherapy plans for prostate cancer patients prior to initiating treatment. At the conclusion of the funding period I have received 10 months of weekly didactic sessions in radiation physics and radiation biology. Both of these topics will be critical to my performance as a practicing clinician and researcher. In addition, understanding the core concepts presented in these sessions is required for certification in the discipline of Radiation Oncology. I sat for the radiation physics and biology examinations in July 2011 and passed (Reportable Outcomes).

Focus Area 3: Participation in the Prostate Cancer Research Programs at the Arizona Cancer Center.

During the funding period I was involved with the Prostate Cancer Research Programs. I have attended the Prostate Cancer Metastasis and Signaling Group weekly and was able to present a component of my research in this forum in October of 2010. My talk was entitled: “Blocking Integrin Function Combined with Ionizing Radiation for the Eradication of Bone Metastasis”.

The weekly Prostate Program Core Meeting has also been fundamental to my

progress. At this meeting I discuss and review results with my collaborator, Dr. Raymond Nagle. Dr. Nagle is a pathologist with expertise in prostate cancer. I had an opportunity to present my data in this forum prior to attending the IMPaCT conference in March 2011. My talk was entitled “Application of an In Vitro Prostate Organ Culture System to Study the Radiobiology of Intact Human Prostate Tissues”.

Focus Area 4: National Meetings/Seminars.

As a result of the training grant, I have had the privilege to present my data at national meetings. Meetings which I attended and presented an abstract are listed below (Reportable Outcomes):

1. American Society for Radiation Oncology 52nd Annual Meeting. October 2010
2. Department of Defense Innovative Minds in Prostate Cancer Today (IMPaCT) Conference. March 2011

In addition, my work has resulted in additional abstracts presented at national meetings that I could not attend listed below (Reportable Outcomes):

1. Radiation Research Society 56th Annual Meeting. 2010
2. American Association for Cancer Research 103rd Annual Meeting. 2012

Focus Area 5: Career Development Roundtables.

Physician-scientists face a difficult challenge to balance both research and patient care. A core component of this training grant was to attend specific mentoring sessions geared for physician-scientists at both local and national meetings. A list of mentoring/networking sessions is provided below:

1. Translational Medicine, San Francisco, July 2010. This meeting provided insight into cutting-edge translational research as well as targeting early-career investigators with mentoring sessions.
   Mentoring Lunch with M. Celeste Simon, PhD, Abramson Cancer Center, University of Pennsylvania.
2. Molecular Biology in Clinical Oncology, an AACR Educational Workshop, July 2010.
   This Educational workshop provided hands-on training in research techniques, didactic teaching, and a grant writing workshop.
   Mentoring sessions were throughout the program with faculty.
3. Department of Defense Innovative Minds in Prostate Cancer Today (IMPaCT) Conference, March 2011. This meeting had many opportunities for networking. I was able to obtain research and career advice from Dr. Peter Nelson, a principal investigator on the Pacific Northwest Prostate Cancer SPORE.
4. 3B Research Forum: Benchtop to Bedside and Back, Atlanta, May 2011.
   This forum was specific to radiation oncologists addressing the need for continued basic and translational research. It featured a specific educational session entitled “How to survive as a translational scientist”.
5. American Brachytherapy Society Seattle Prostate Fellowship, December 2011.
   The fellowship provided me with hands-on experience with the experts in prostate cancer brachytherapy such as Dr. Kent Wallner and Dr. Peter Grimm.

Both have published extensively in the field. I was able to obtain career advice from both a clinical and research perspective.

Research Program:

Aim 1, Task 1:

As reported in the annual summary (1) the initial phase of the research involved learning the technical skills to perform prostate organ culture as well as optimizing the conditions to maintain appropriate viability in the tissue slices. The final media conditions included Medium 199 supplemented with 5% FBS, 1mM dihydrotestosterone, 0.1mM dexamethasone, 2mg/ml insulin, 200mM glutamine, 250mg/ml fungizone, 50mg/ml gentimycin, and 100U/ml penicillin/streptomycin. Using the dynamic roller culture system, conditions were optimized and we found that the normal epithelial and cancer components of the tissue slices were maintained at maximum for five days in culture, however at that point there was significant heterogeneity within the slice in terms of viability. This finding is in contrast to other groups reporting viability for up to 14 days (2). Although effort was placed on continued optimization of the culture conditions, we have not found a formulation that will reliably preserve the tissue for more than three days. Therefore it was determined that the optimal timeframe to conduct the proposed experiments would be within three days of initiating culture.

As demonstrated in Figure 1 and 2 (Supporting Data), these conditions allowed cultured normal and malignant prostate epithelium to retain tissue-specific markers such as cytokeratin 5/14 and prostate-specific antigen. In addition, proliferation of normal prostate basal cells was observed while prostate cancer tissues lost the proliferative marker, Ki67, while in culture. Although ideally we would like to maintain the indolent features of human prostate tissue, our media formulation at this time promotes growth of the normal glands. Additional study is ongoing to further optimize media conditions to mimic prostate tissue characteristics *in vivo*.

Aim 1, Task 2:

As reported above in Aim 1, Task 1, the optimal timeframe to conduct the experiments involving ionizing radiation would be within three days of initiating culture due to a decrease in tissue viability beyond 3 days. We reported in the prior annual summary (1) that this time limitation in culture altered our approach to comparing the DNA damage response to conventional and hypofractionated radiation. The methodology we used for irradiation experiments includes the following: Following Day 1 in culture, tissue slices were irradiated with a single fraction of 2 or 8 Gy. Slices were fixed in formalin and paraffin-embedded at 1, 8, 24, and 48 hours after radiation treatment. Immunohistochemistry was performed to evaluate for phosphorylation of H2AX on serine 139 (gamma-H2AX), p53, and Rad51 using DAB chromogen.

Figure 3 (Supporting Data) demonstrates that normal prostate epithelial components display early and late markers of the DNA damage response following irradiation with 8 Gy. The predicted timing of repair proteins were consistent with the

literature with the early marker of DNA double-strand breaks, gamma-H2AX, responding at 1 hour post-irradiation (Figure 3A) while Rad51, a protein involved in homologous repair, was not induced until 8 hours post-irradiation (Figure 3B). As predicted, the 8 Gy treated tissues had an amplified DNA damage response in comparison to the 2 Gy samples (data not shown). It was also observed that the DNA damage response predominated in the basal cell layer of the epithelium (Figure 3A & B) which is in agreement with others (3).

Since the annual summary we have been able to study the DNA damage response in prostate cancer tissues. As demonstrated in Figure 4 (Supporting Data) prostate cancer epithelium responds to ionizing radiation with the predicted early marker for DNA damage, gamma-H2AX foci. Interestingly, there is a high level of baseline positivity for gamma-H2AX suggesting genomic instability of the tumor can be detected by this method. In addition, in contrast to the response of normal prostate epithelium described above, prostate cancer tissue did not demonstrate induction of Rad 51 positivity over time (data not shown). This may be explained by the finding that in our culture system the normal glands begin to proliferate and therefore would be more likely to utilize the homologous (HR) pathway for repair. The prostate cancer tissues remain non-proliferative in culture suggesting the predominant pathway could be non-homologous end joining (NHEJ).

Part of this aim was to determine if there was a predominant pathway for repair by staining for Rad51 and Ku 70/80 to differentiate between HR and NHEJ respectively. Unfortunately, in our hands, commercially available antibodies for Ku 70/80 did not show a difference between irradiated and non-irradiated tissue. We feel this is a technical problem with the antibody and we will continue to test for an appropriate marker in prostate tissue to determine if the NHEJ pathway of repair is active.

The limitation of the time the tissue remains viable (Aim 1, Task 1 discussed above) has precluded us to this point in determining if hypofractionated ionizing radiation has a unique damage response signature in comparison to conventionally fractionated radiation. In order to deliver a biologically equivalent fractionation schedule for comparison, the tissue would need to remain viable for at least one week. This remains a clinically relevant question and will be explored as our culture techniques improve.

Aim 1, Task 3:

As discussed above, the damage response in prostate cancer tissue demonstrates the early marker for DNA double-strand breaks, gamma-H2AX (Figure 4). Our technique for organ culture involves taking a core from the transition zone of a prostatectomy specimen. We do not take a core from the peripheral zone as this could possibly compromise pathologic analysis. Therefore, we often do not obtain prostate cancer in our tissue sample. Currently we have only obtained specimens with Gleason 3+3=6 disease and cannot determine if Gleason score has an impact on our endpoints for damage. This remains an interest and has clinical relevance. With continued collection of prostatectomy samples this aim will eventually be determined.

Aim 1, Task 4:

As noted in the prior annual summary, each experiment creates a tissue repository for further study which is currently available for creation of a tissue microarray. The microarray of irradiated prostate tissue will be explored further as more prostate cancer specimens are collected.

Aim 1, Task 5:

The power of the organ culture system is that the complex tissue architecture remains intact. We wanted to study if the microenvironment, specifically the cell adhesion status of prostate tumor cells, influenced the survival signaling following ionizing radiation. We therefore investigated in prostate cell lines whether a radiation induced survival response (measured by AKT activation) was modified by altering alpha-6 integrin function or by supplying laminin-5 (Laminin 332), a native basement membrane protein. Our results indicate that prostate cancer cells that reside on laminin-5 following irradiation have an amplified phosho-AKT response as compared to cells residing on a tissue culture surface (Figure 5). Furthermore, altering integrin function through integrin cleavage blocks the IR dependent phospho-AKT response (Figure 6). A model demonstrating how integrin cleavage can modulate cell adhesion and integrin dependent cellular responses is shown in Figure 7. These data suggest that cell adhesion mediated radiation resistance may play a role in prostate cancer through alpha-6 integrin interactions. It is an important future area for study within the organ culture system to demonstrate if this phenomenon may be clinically relevant.

Aim 1, Task 6:

As demonstrated in Aim 1, Task 5 and by others, the phenomenon of cell adhesion mediated radiation resistance is in part dependent on integrin mediated survival signals. In addition, integrins have been shown to be involved in radiation induced tumor cell invasion and migration (4). To determine if altering integrin function (Figure 7) could block radiation induced cell migration, we utilized a wound healing assay and exposed prostate tumor cells that stably expressed the non-cleavable cell surface mutant of A6B1 integrin (PC3N-RR) or the wild type integrin (PC3N-WT) to sub-lethal doses of radiation (1 or 2 Gy). Interestingly, laminin-5 (LN-332) suppressed radiation induced cell migration in tumor cells with the wild-type A6 integrin (Figure 8) while the A6 non-cleavable mutant (PC3N RR) removed laminin-5 suppression of radiation-induced prostate tumor cell migration (Figure 9). These data suggest that the A6B1/laminin-5 adhesion event suppresses radiation induced cell motility and is an area of interest to understand the mechanism of radiation induced cell migration.

Aim 1, Task 7:

Although radiotherapy is an effective definitive therapy for prostate cancer, there are failures. The goal of this task was to determine if the DNA damage response was influenced by the invasive phenotype of a human prostate cancer cell line. The endpoint chosen was the production, resolution, and persistence of gamma-H2AX foci as an indicator of damage, repair, and inadequate repair respectively. Figure 10 demonstrates a dose-dependent increase in residual DNA damage and genomic instability in DU145 cells.

Several groups have shown that retention of gamma-H2AX foci is an indication of lethal DNA damage (5). When the cells are stimulated into a migratory phenotype with hepatocyte growth factor (HGF) there is an amplification of residual DNA damage (Figure 11). These data suggest that an invasive tumor phenotype inadequately repairs DNA damage following exposure to ionizing radiation. An important future direction currently being pursued is to determine if indeed the residual DNA damage predicts tumor cell survival. If residual gamma-H2AX foci can predict tumor cell survival, it would be a fantastic endpoint to use in the organ culture model system to study dose and fractionation effects on human prostate tissue.

Aim 2, Task 1:

We reported in the annual summary that there were technical difficulties with retaining viability of the stromal tissue in culture. This is can be demonstrated by viewing hemotoxylin and eosin stained specimens after organ culture which display a loss of stromal cell viability throughout the specimen (Figures 1 &2, H&E panels).

This technical problem limits the ability to study the damage response within the stromal tissue following irradiation. Other groups using the organ culture system have not reported this problem (3). Unfortunately they have not published their media formulation and have not responded to our requests. We will continue to optimize our conditions so this aim can be performed.

Aim 2, Task 2:

There are technical difficulties with retaining viability of the stromal tissue in culture as outlined in Aim 2, Task 1 which makes this task difficult to perform. We are continuing to work to eliminate this technical problem.

Aim 2, Task 3:

Each experiment creates a tissue repository for further study. Pending elimination of technical issues with retaining stromal tissue, a microarray can be generated.

Aim 3, Tasks 1-3:

This aim was crafted with the intent to provide clinically translational results from this study as the kinetics of the biochemical response of prostate specific antigen (PSA) is monitored closely following definitive radiotherapy and has been shown to have prognostic significance (6). We have shown previously that PSA is secreted and can be measured by ELISA in the prostate organ culture (7). As discussed above (Aim 1, Tasks 1-2), the organ culture system has limitations beyond three days. This precludes us to comparing biologically equivalent fractionation schedules (hypofractionated vs conventional fractionation). In order to deliver a biologically equivalent fractionation schedule for comparison, the tissue would need to remain viable for at least one week. Therefore, although conditioned media from Specific Aims 1 and 2 have been collected, PSA specific ELISAs were not performed as they would not give any meaningful data other than show a

dose response for curve which is known a priori. This aim remains a relevant question with clinical implications and will be explored as our culture techniques improve.

KEY RESEARCH ACCOMPLISHMENTS:

Training Program:

- Four abstracts presented at national meetings.
- Specialized training in prostate brachytherapy.
- Board Eligibility obtained in Radiation Oncology
- Manuscript in preparation: “Inadequate repair of ionizing radiation damage: a consequence of the invasive tumor phenotype”
- Grant in preparation: “Prostate cancer perineural invasion and radiation resistance” Department of Defense Prostate Cancer Physician Training Award
- Employment as an Assistant Professor at Dartmouth-Hitchcock Norris Cotton Cancer Center

Research Program:

- Normal prostate tissue and prostate cancer can be cultured *ex vivo* using a dynamic organ culture system.
- Tissue-specific markers such as cytokeratin 5/14 and prostate specific antigen are retained in culture.
- Proliferation of normal prostate basal cells are observed in culture while the normal stromal component cannot be maintained.
- Normal prostate tissue responds to ionizing radiation with predicted repair foci (gamma-H2AX and Rad51) and a marker for cellular stress, p53.
- The DNA damage response in normal prostate tissues appears to predominate in the basal cell layer.
- Prostate cancer epithelium responds to ionizing radiation with predicted early repair foci (gamma-H2AX) but does not display late homologous recombination foci (Rad51).
- Prostate tumor cell adhesion to laminin-5 after irradiation will result in amplification of a pro-survival signal (phospho-AKT).
- Cleavage of the A6 integrin subunit will block the irradiation induced pro-survival signal (phospho-AKT)
- Residual DNA damage, detected by persistent gamma-H2AX foci, is amplified by an invasive tumor cell phenotype.

REPORTABLE OUTCOMES:

Training Program:

Employment:
Assistant Professor of Medicine, Section of Radiation Oncology.
Dartmouth-Hitchcock Norris Cotton Cancer Center
Hanover, New Hampshire
Board Eligible, Radiation Oncology

Ultrasound-guided Transperineal Brachytherapy for Prostate Cancer
Training Course, Seattle Prostate Institute, Swedish Medical Center, Seattle WA.

High Dose-Rate Brachytherapy for Prostate Cancer Training Course, Seattle Prostate Institute, Swedish Medical Center, Seattle WA.

American Association for Cancer Research Workshop, Molecular Biology in Clinical Oncology, The Given Institute, Aspen CO.

American Brachytherapy Society Seattle Prostate Fellowship

Research Program:
Grants:
In Preparation: Sroka TC and Cress AE. "Prostate cancer perineural invasion and radiation resistance" Department of Defense Prostate Cancer Physician Training Award

Abstracts:
Pond E, Sroka TC, Gard J, Nagle, RB, and Cress AE. Inadequate repair of ionizing radiation damage: a consequence of the invasive tumor phenotype. *American Association for Cancer Research 103rd Annual Meeting.* 2012

Sroka TC, Gandolfi AJ, Nagle RB, Lutz W, Sokoloff MH, and Cress AE. Application of a prostate organ culture system to study the radiobiology of intact human prostate tissue. *Department of Defense Innovative Minds in Prostate Cancer Today (IMPaCT) Conference.* 2011

Sroka TC, Pawar SC, Pond GD, Nagle RB, and Cress AE. Blocking integrin function combined with ionizing radiation for eradication of bone metastasis. *American Society for Radiation Oncology 52nd Annual Meeting. 2010*

Sroka TC**,** Cameron R, Nagle RB, and Cress AE. Overcoming cell adhesion mediated radiation resistance by altering A6B1 integrin function. *Radiation Research Society 56th Annual Meeting. 2010*

CONCLUSION:
Training Program:
My ultimate career goal is to contribute to the field of prostate cancer research and therapy as an accomplished academic physician. The training plan has provided mentor guidance with an established prostate cancer researcher and enabled networking with both clinical and basic science colleagues interested in prostate cancer research. In addition, it has fostered my development as both a clinician and a scientist through specialized training in prostate brachytherapy and by securing a competitive

fellowship at a national research workshop for early-career investigators. This award has been instrumental in obtaining employment as an academic radiation oncologist at Dartmouth-Hitchcock Norris Cotton Cancer Center. It has also equipped me with the skills to continue my research aspirations and future grants are in preparation. Therefore, this award has provided specific training for managing both clinical and independent research skills necessary to develop a career in translational prostate cancer research.

Research Program:

The aim of this proposal was to determine the differences in radiobiological response of human prostate tissue to conventional and hypofractionated radiotherapy. Specifically, this proposal characterized the predominant DNA damage response pathway from human prostatectomy specimens in response to a conventional or hypofractionated dose of ionizing radiation. We have shown that normal prostate tissue and prostate cancer can be cultured *ex vivo* using a dynamic culture system and used to study the radiobiology of human prostate tissue. Normal prostate tissue responds to ionizing radiation with predicted repair foci (gamma-H2AX and Rad51), and a marker for cellular stress, p53. The DNA damage response in normal glands appears to predominate in the basal cell layer. Prostate cancer epithelium responds to ionizing radiation with predicted early repair foci, gamma-H2AX, but in contrast to normal prostate epithelium, homologous repair foci (Rad51) were not demonstrated. Identifying if the non-homologous end-joining (NHEJ) repair pathway is predominant in prostate cancer epithelium is a focus of study using the organ culture model system.

We have shown with prostate cancer cell lines that pro-survival signals via AKT may play a role in cell adhesion mediated radiation resistance (CAM-RR). This signal is in part dependent on the A6 integrin subunit. It is of interest for future studies to test if radiation induced A6 dependent pro-survival signals are demonstrated in the *ex vivo* culture system. Demonstrating that this phenomenon is present in intact human prostate tissues may further substantiate its clinical relevance and ultimately lead to new therapies.

Clinically relevant research is fundamental for advances in cancer therapies. The research plan crafted had direct application to problems in prostate cancer radiotherapy currently being investigated in clinical trials. The short-term viability of the *ex* vivo prostate tissues precluded us from testing a biologically equivalent fractionation schedule to test if there is a unique damage response signature between hypofractionated and conventional radiation. We plan to continue optimize the organ culture conditions to mimic the indolent nature of prostate tissues and to retain the integrity of the specimen. This will allow us to test this clinically relevant question and continue to develop this system for translational prostate cancer research.

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APPENDICES:

None

SUPPORTING DATA:

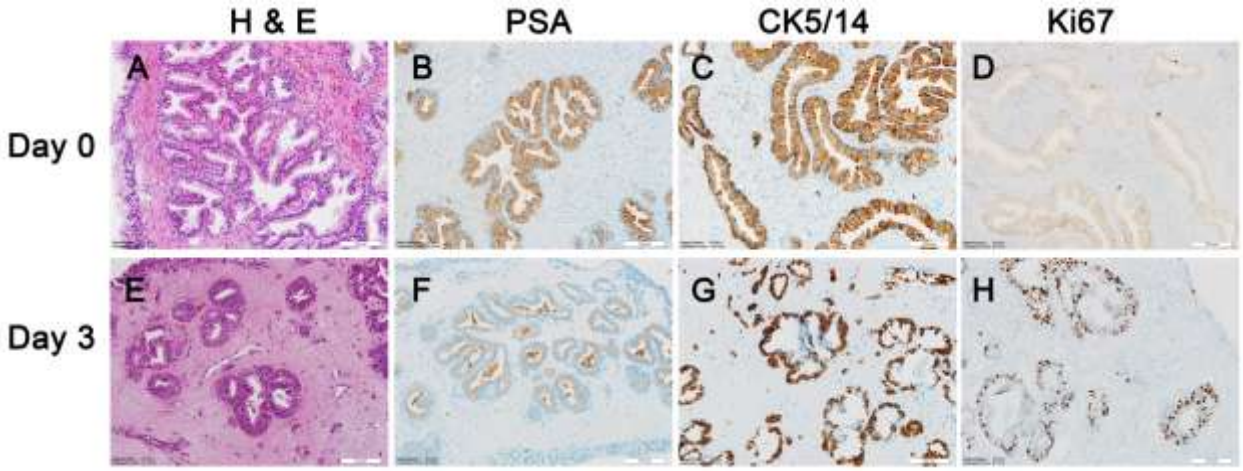


Figure 1. Cultured normal prostate tissues retain tissue-specific markers and demonstrate basal cell proliferation. Precision-cut slices were generated from the transitional zones of human prostate specimens . They were cultured in Medium 199 supplemented with 5% FBS, 1mM dihydrotestosterone, 0.1mM dexamethasone, 2mg/ml insulin, 200mM glutamine, 250mg/ml fungizone, 50mg/ml gentimycin, and 100U/ml penicillin/streptomycin. Slices were cutured for 3 days. Slices were fixed in formalin and paraffin-embedded. Slices were stained with hemotoxylin and eosin (A &E) and immunohistochemistry was performed to evaluate for PSA (B&F), cytokeratin 5/14 (C&G) and Ki67 (D&H) using DAB chromogen.

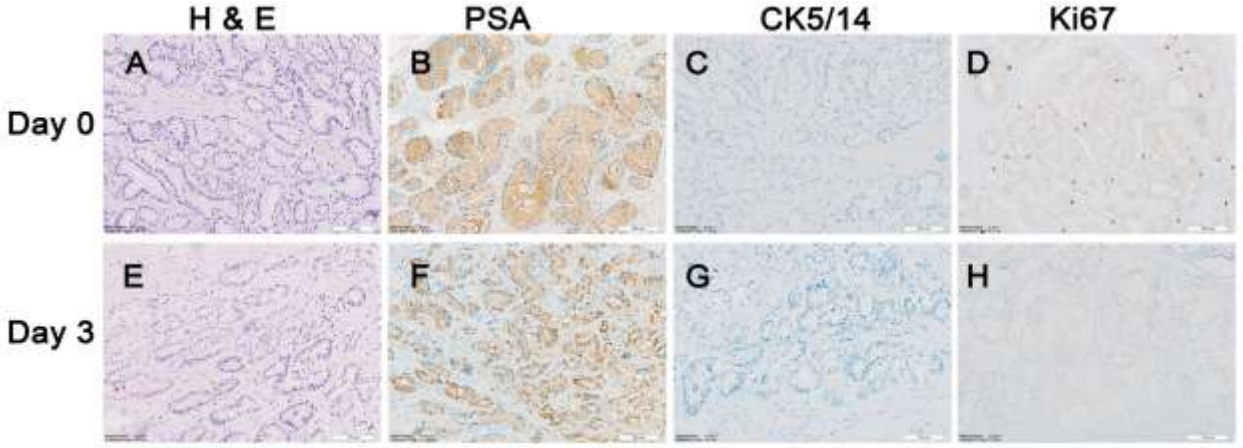


Figure 2. Cultured prostate cancer tissues retain tissue-specific markers and cease proliferation. Tissues were cultured as described in Figure 1. Slices were stained with hemotoxylin and eosin (A &E) and immunohistochemistry was performed to evaluate for PSA (B&F), cytokeratin 5/14 (C&G) and Ki67 (D&H).

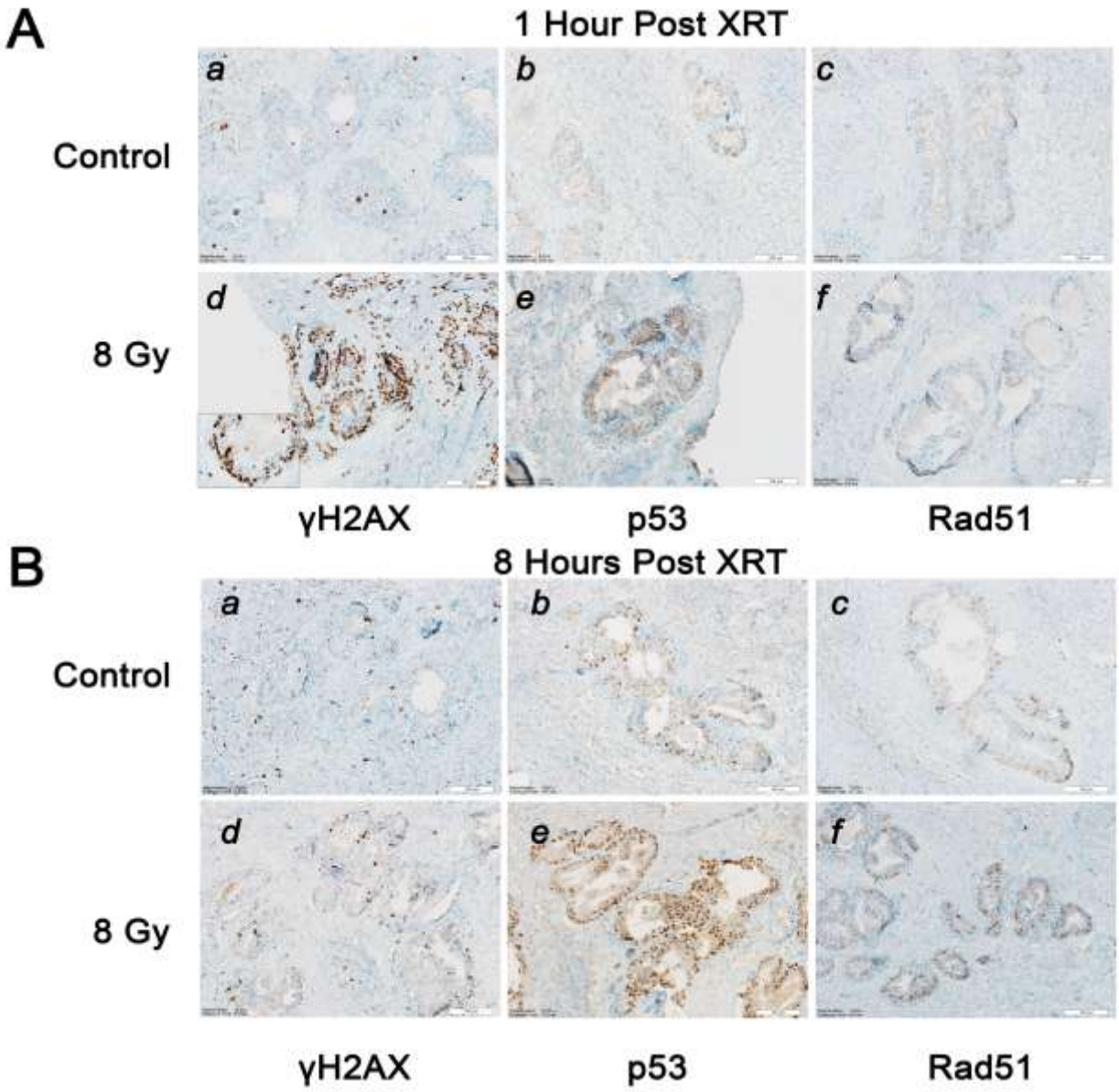
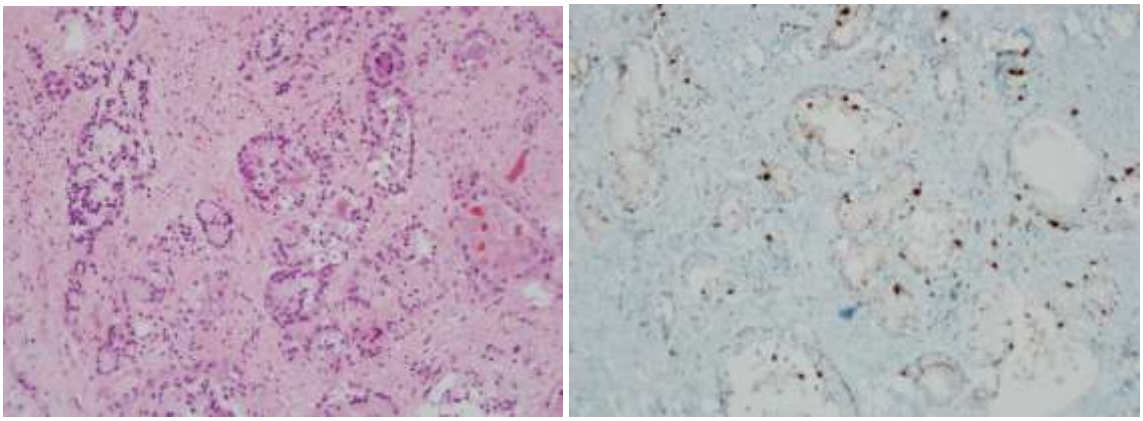


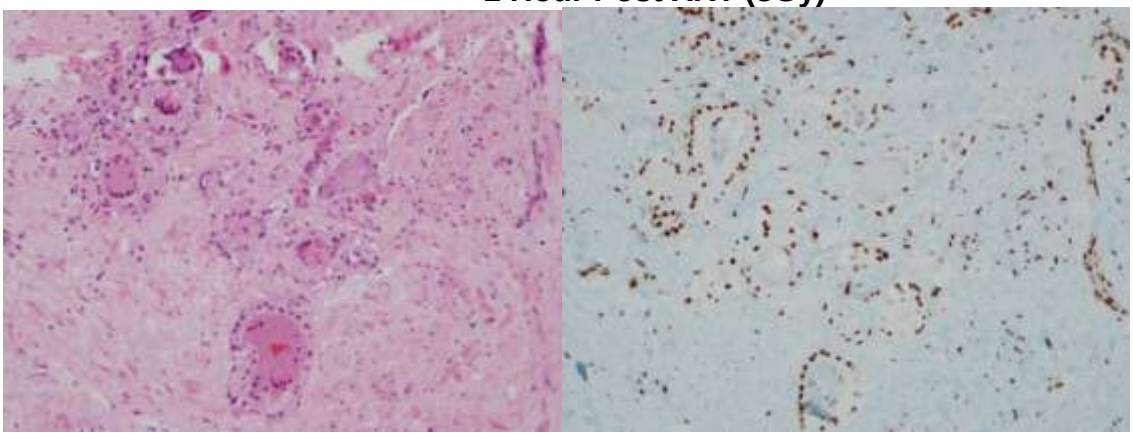
Figure 3. Cultured normal prostate tissues demonstrate early and late markers of the DNA damage response following treatment with ionizing radiation. Tissues were cultured as described in Figure 1. After 1 day in culture they were irradiated with a single fraction of 8 Gy. Slices were fixed in formalin and paraffin-embedded 1 hour (A) or 8 hours (B) after radiation treatment. Immunohistochemistry was performed to evaluate for phosphorylation of H2AX on serine 139 (γH2AX, a&d), p53 (b&e), and Rad51 (c&f) using DAB chromogen.

Control



H&E **γH2AX**

1 Hour Post XRT (8Gy)



H&E **γH2AX**

Figure 4. Cultured prostate cancer tissues demonstrate an early marker of the DNA damage response following treatment with ionizing radiation. The tissues were cultured as described in Figure 1. After 1 day in culture they were irradiated with a single fraction of 8 Gy. Slices were fixed in formalin and paraffin embedded 1 hour after radiation treatment. Immunohistochemistry was performed to evaluate for phosphorylation of H2AX on serine 139.

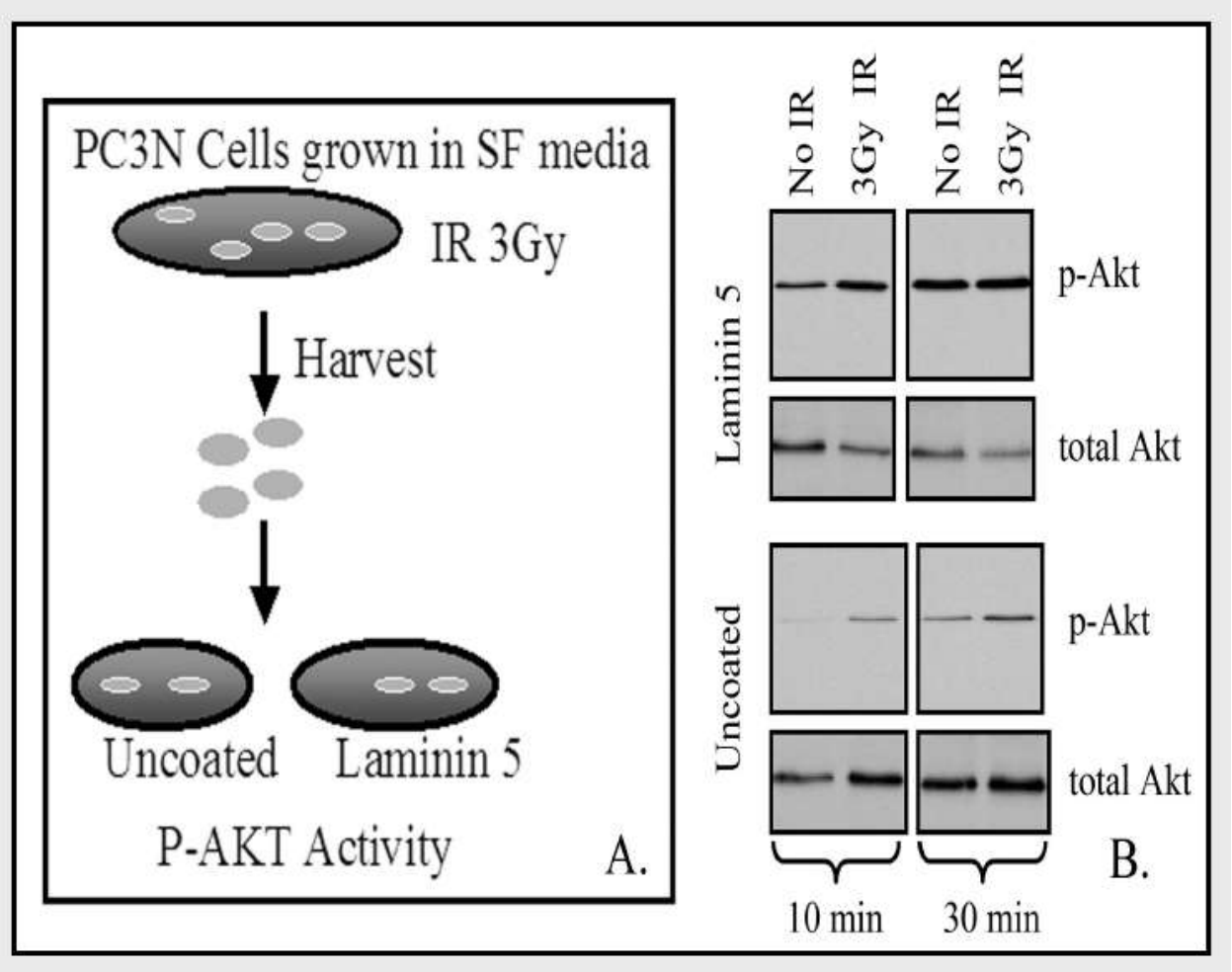


Figure 5. Laminin-5 adhesion amplifies the P-AKT response. PC3N cells were grown in serum free media and irradiated with 3 Gy. They were then harvested and plated onto uncoated or laminin-5 coated dishes. P-AKT and Total AKT were detected by western blot analysis.

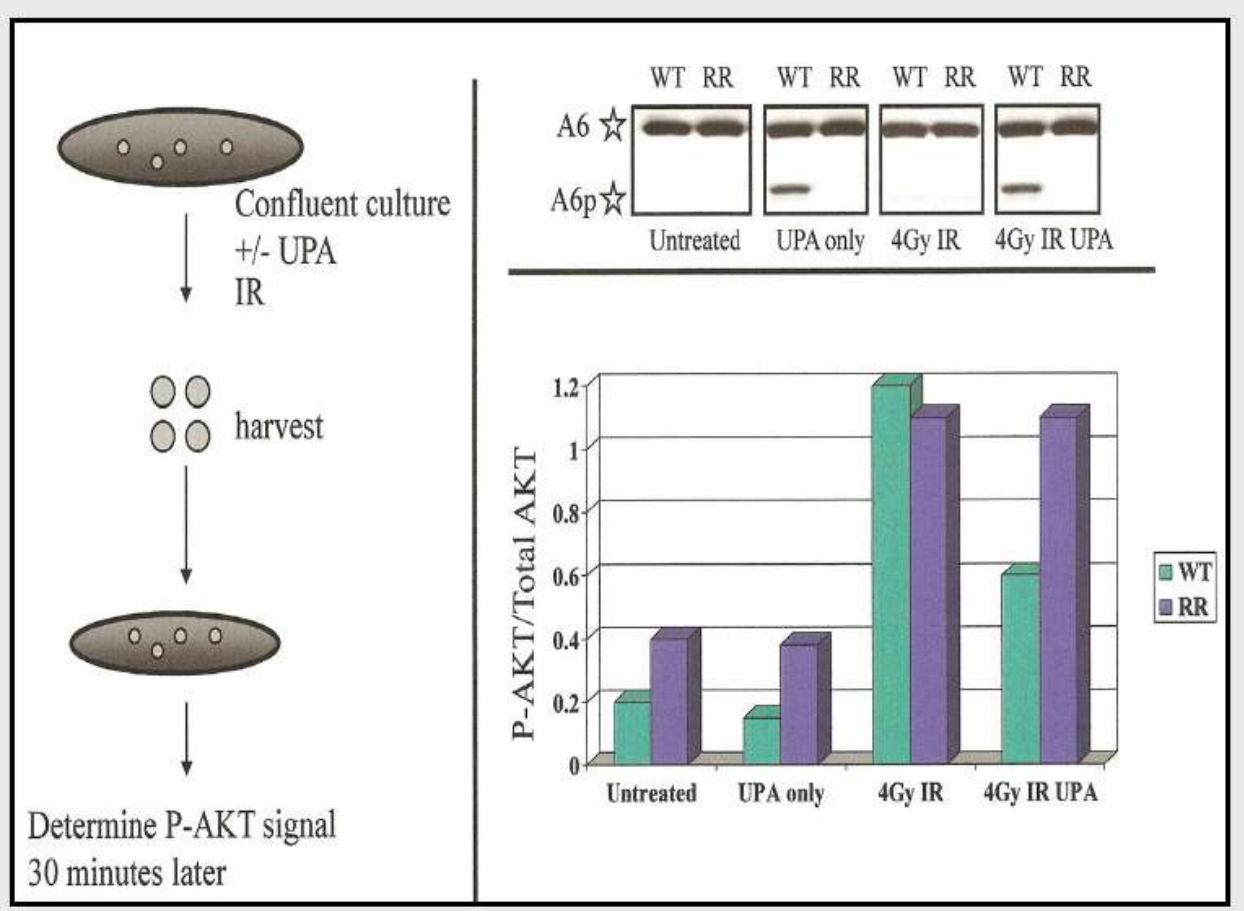


Figure 6. Integrin Cleavage Blocks the IR Dependent P-AKT Response. PC3N cells expressing either the wildtype integrin (WT) or the uncleavable RR mutant (RR) were irradiated with 4 Gy in the presence or absence of UPA. Cells were harvested and placed on laminin coated dishes and the presence of the full length integrin (A6, star), the cleaved integrin (A6p, star), and the P-AKT/Total AKT signals were determined by western blot.

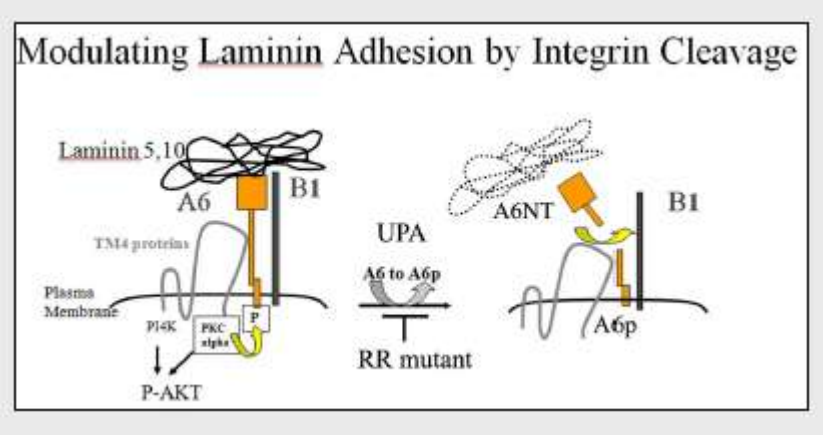


Figure 7. Removal of Laminin Binding Domain by Integrin Cleavage and a Genetic Approach to Block Cleavage. The A6B1 integrin binds to laminin-5 (LN 332) via the N-terminal domain. The A6 integrin subunit is cleaved via urokinase (UPA), removing the laminin binding domain. Integrin cleavage can be induced by exogenous addition of UPA or can be blocked by genetically modifying the integrin cleavage domain using PC3N-RR mutants.

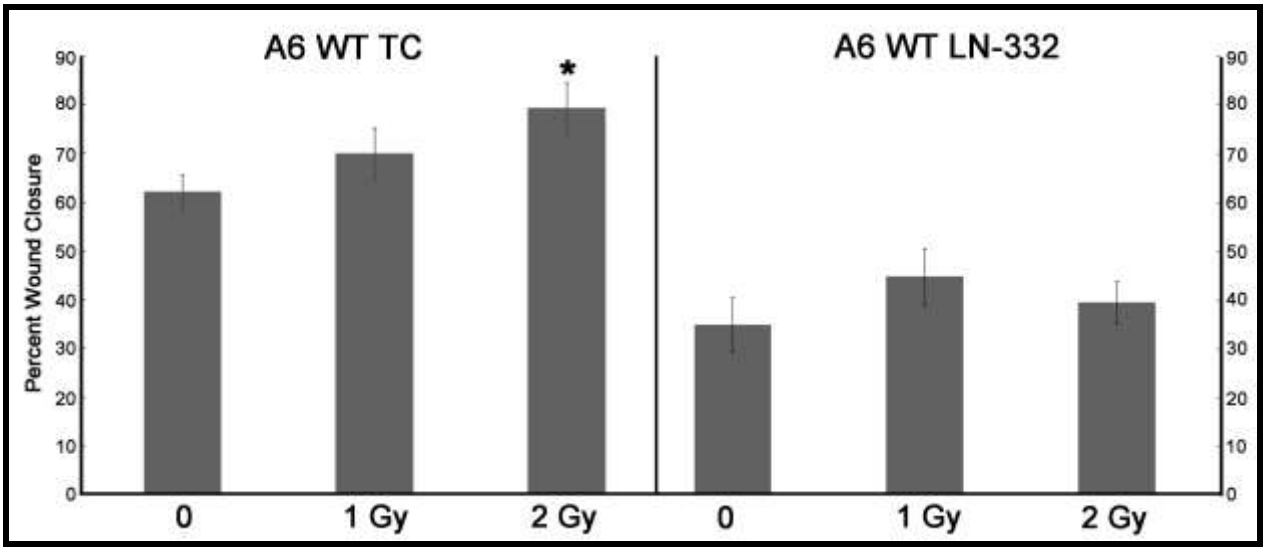


Figure 8. Suppression of radiation-induced prostate tumor cell migration by laminin-5 (LN-332). The wound healing assay was performed using PC3N A6 wild-type cells (A6 WT) that were grown to confluence on tissue culture plastic (TC) or laminin-5 from HaCaT conditioned medium. A scratch was made with a cell scraper and each initial wound was imaged at 12 distinct points. Cells were untreated or irradiated with sub-lethal doses of 1 or 2 Gy and cultured in the presence of 1%FBS for 12 hours prior to analysis for extent of wound closure as described in Figure 3. (**Left panel)** Irradiation enhances PC3N (A6 WT) prostate tumor cell migration on tissue culture plastic, *p-value <0.05. **(Right panel)** Adhesion to laminin-332 blocks radiation-induced PC3N (A6 WT) prostate tumor cell migration.

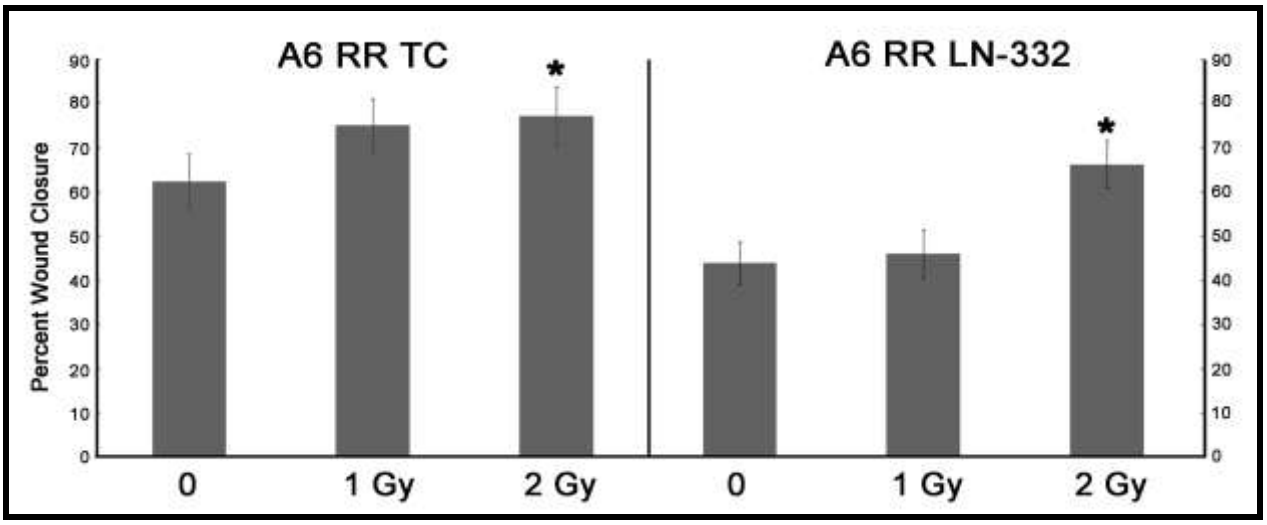


Figure 9. The A6 non-cleavable mutant (PC3N RR) removes laminin-5 (LN-332) suppression of radiation-induced prostate tumor cell migration. The wound healing assay was performed on PC3N RR cells as described in Figure 8. (**Left panel)** Irradiation enhances PC3N (RR) prostate tumor cell migration on tissue culture plastic. **(Right panel)** Adhesion to laminin-5 enhances cell migration in prostate tumor cells with the A6 non-cleavable mutant (PC3N RR). * $p < 0.05$.

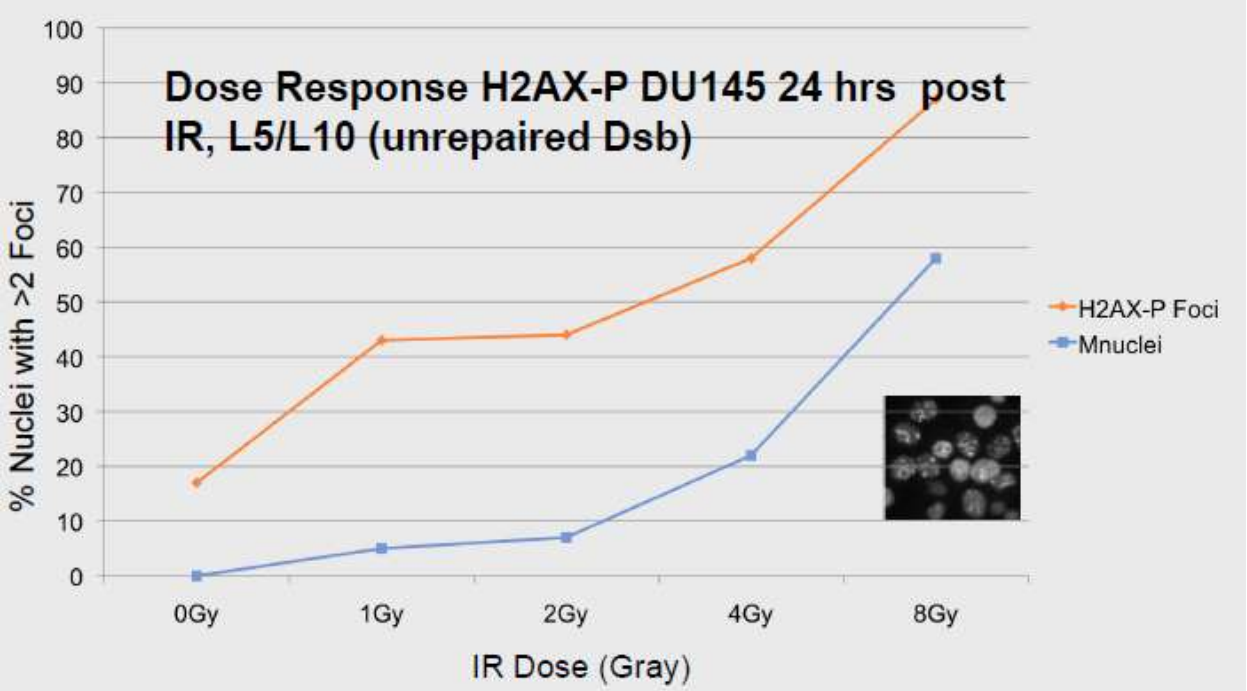


Figure 10. Dose Dependent Residual DNA Damage and Genomic Instability in Response to Ionizing Radiation. DU145 cells were irradiated with 1-8 Gy, and analyzed for the persistence of DNA damage (H2AX-P Foci) or micronuclei (Mnuclei) by immunohistochemistry 24 hours post radiation. Cells in G2 or mitosis were excluded.

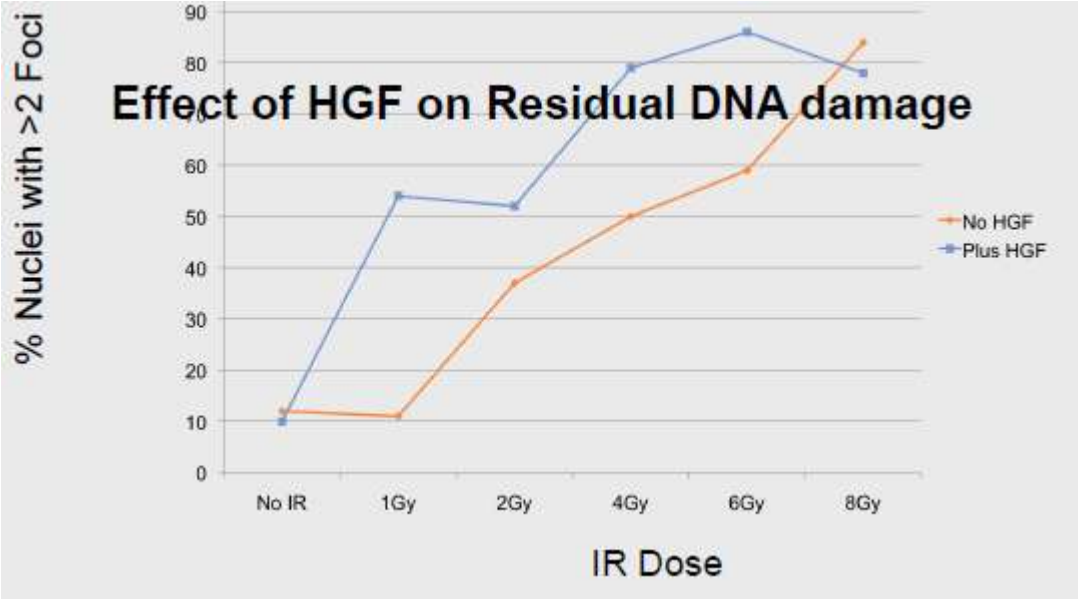


Figure 11. Dose Dependent Residual DNA Damage is Amplified by the HGF-Induced Cell Motility. DU145 cells were grown without (No HGF) or with 5ng/ml HGF (Plus HGF) for 18 hours prior to irradiation. Residual DNA damage was detected 48 hours post radiation by quantifying the percentage of nuclei containing >2 foci (Y-axis) using immunohistochemistry images. Cells in G2 or mitosis were excluded.